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K.9 Expression of CD2R on T cells of rheumatoid arthritis patients

A. J. Potocnik[1], H. Menninger[2], F. Emmrich[1], and R. A. Kroczek[1]

Activated T cells seem to play an important role in the perpetuation of the inflammatory process in rheumatoid arthritis (RA). One surface molecule known to contribute to T cell activation *in vitro* is CD2. Upon stimulation of T cells a hidden epitope designated CD2R is unmasked, which is a pre-requisite for signal transduction via CD2. Although the requirements for triggering via CD2 are well established *in vitro*, curiously no data were reported on the expression of CD2R *in vivo*. The aim of our study was to investigate CD2R expression in healthy individuals and RA patients using 4 distinct monoclonal antibodies (mabs) [T11-3, 9-1, Vit 13, D 66] applied in two-color flow cytometry. First we studied CD2R expression on peripheral blood (PB) T cells of healthy individuals and on spleen and tonsil T cells of control donors. In 5 out of 6 PB T cell samples staining with mabs T11-3, 9-1 or Vit 13 was weak or absent, whereas one healthy donor was clearly positive. In spleens ($n = 3$) and tonsils ($n = 2$) we found 10–15 % of all T cells positive for CD2R. In RA patients we could demonstrate a very high percentage (65–95 %) of $CD2R^+$ cells on PB and synovial fluid (SF) T cells. Only 1 out of 9 patients showed a weak CD2R staining. The intensity of staining with CD2R on SF T cells was typically higher than on PB T cells. Mab D 66 showed a different staining pattern in all tissues. Our main results can be summarized as follows: i) the CD2R epitope is strongly expressed on PB and SF T cells of RA patients; ii) mabs T11-3, 9-1, Vit 13 versus D 66, all clustered to recognize the CD2R antigen, apparently detect different epitopes on this molecule.

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K.10 Fine specificity of *Yersinia enterocolitica* reactive synovial fluid T-cell clones in Yersinia arthritis

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Yersinia enterocolitica enteritis may be followed by reactive arthritis in genetically predisposed patients. T-cell mediated immune responses seem to be involved in the pathogenesis of Yersinia arthritis. In this study the fine specificity for different bacterial antigens of a panel of synovial fluid (SF)-derived T lymphocyte clones (TLC) was analyzed. From the SF of two patients (patient HD, HLA $B27^-$, HLA $DRw6^+$; patient JP, HLA $B27^+$, $DR2,4^+$) with Yersinia arthritis, T cell lines were established. The lines were subcloned and the proliferative responses of the resulting TLC to different *Y. enterocolitica* cell fractions were investigated. Bacterial cell fractions were prepared by differential centrifugation of desintegrated *Y. enterocolitica*. Inner and outer membrane fractions were separated by sucrose density gradient centrifugation, cytoplasmic fractions by differential ammonium sulfate precipitation and

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desalting of the macromolecules by gelfiltration with Sephadex G25. The bacterial cell fractions were analyzed by sodium dodecyl sulfate-polyacrylamid gel electrophoresis. With the T-cell line from patient HD the best cloning efficiency was achieved with the membrane fraction as the stimulating antigen, in the case of patient JP with the cytoplasmic fraction. Whereas several clones of patient JP showed crossreactivity between *Y. enterocolitica* and *Salmonella typhimurium*, essentially all clones from patient HD were specific for *Y. enterocolitica* antigens. The fine specificity of $CD4^+$ TLC, proliferative responses of which could be blocked by protein digestion, was further investigated. TLC of patient JP differed in the recognition of cytoplasmatic macromolecular ammonium sulfate precipitated fractions (CMAPF). Some clones could also be stimulated by the cell envelope of *Y. enterocolitica*. Experiments with TLC of patient HD showed greatest stimulation indices in response to membrane antigens. Several TLC with different specificities for either inner or outer membrane preparations and to CMAPF could be propagated. In conclusion, SF-derived T-cell clones from patients with Yersinia reactive arthritis represent a multiclonal response to various protein antigens of *Y. enterocolitica*. This response differs in different individuals and may induce or maintain synovitis.

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K.11 A fluorescent lectin-agarose bead immunoassay for a pancreatic autoantigen involved in Crohn's disease

Wiebke Rögener, E. Horbach, Doris Kaiser, W. Stöcker, M. Otte, and P. C. Scriba

Pancreatic autoantigen (PAg) which is recognized by many sera of patients with Crohn's disease (CD) could up to now only be identified by a neutralization technique, in which the capacity of samples to extinguish a specific immunofluorescence reaction of CD-sera with human pancreatic tissue sections was recorded. However, the neutralizing efficacy of crude duodenal juice or homogenized pancreas could not be evaluated, since activated proteinases digested the tissue sections. Solid phase immunotests, based on direct binding of PAg to plastic surfaces gave inconsistant results, possibly depending on the low affinity of PAg to the materials used. We have found out that PAg binds to soybean agglutinin (SBA) which is known to react with N-acetylgalactosamine. Thus, it was possible to develop a new assay for the detection of PAg: Samples were diluted in microtiter plates (50 µl per well) and incubated for 15 min with 10 µl of a 50% suspension of SBA-coated agarose beads (Camon, Wiesbaden). 50 µl of 1% ovalbumine were added. After washing in PBS, beads were further incubated with 50 µl of HL-1 (monoclonal antibody against PAg; for 30 min, washed again and stained for 30 min with FITC-labelled goat anti-mouse IgG. Results were read with a fluorescence microscope. Positive beads exhibited a bright fluorescence. Of 200 samples which were identified to contain PAg as determined by the neutralization test, each was clearly positive in the lectin agarose bead immunoassay. Semiquantification was possible by titration of samples in twofold dilutions, which resulted in a clear cut end point. PAg could easily be detected in homogenized pancreas and contents of the bowel. The new assay for PAg was simple to perform, easy to read and seems to be more widely applicable than the neutralization test.

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K.12 Frequencies of antigen-specific B cell precursors in patients with rheumatoid arthritis

Ulla Rudolphi and Inga Melchers

In the present study frequencies of antigen-specific B cells from patients with rheumatoid arthritis (RA) are investigated. For this purpose we used the El-4 culture system (1) that allows a very potent stimulation of human B cells. B cells were prepared from the peripheral blood of patients and control persons by rosetting and phenotyped using antibodies to CD19, CD20 and surface immunoglobulins (Ig). Then the cells were incubated in various concentrations together with a mutant subclone of the mouse thymoma line El-4 and T cell/macrophage supernatants, which stimulate human B cell precursors for activation, proliferation, and differentiation. Ig concentrations and antibody-specificities were analysed by ELISA in culture supernatants collected after 10 days. Antibody secreting B cell clones generating IgM, IgG, and IgA occurred in frequencies of 1/1.1, 1/2 and 1/2.2, respectively. The mean amount of IgM and IgG per B cell clone was 58 ± 26 ng and 41 ± 34 ng. Recently, relatively high frequencies of B cell precursors generating IgM with specificities for IgG-Fc fragments, various collagens (kindly provided by K. von der Mark, Erlangen) and the 65 kD heat shock protein (hsp65) of *M. tuberculosis* (kindly provided by S. H. E. Kaufmann, Ulm) were found in patients with RA and controls. In contrast, hsp65-specific B cell precursors secreting IgG or IgA could only be determined in patients with RA (controls: < 1/3000, patients: 1/450–1/1200).

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K.13 Common antigens between *Mycoplasma arthritidis* and host tissue – a possible basis for autoimmune reactions in chronic arthritis

Martin Runge, Karin Busche, Michael Schlesier, Hong Bum Koh, Dorothea Spaller, and Helga Kirchhoff

Previous investigations demonstrated common antigens between *Mycoplasma* (M.) *arthritidis*, the causative agent of a severe polyarthritis in rats, and several rat cells, including joint chondrocytes. These antigens have been characterized with polyclonal and monoclonal antibodies in EIA, SDS-PAGE and Western blot and demonstrated in light and electronmicroscopy by immuno-gold-silver-staining. It appeared that polyclonal as well as monoclonal antibodies were reacting with the cartilage matrix. The reactive component of the human cartilage matrix turned out to be a large aggregating proteoglycan. The crossreacting antigens of the *M. arthritidis* membrane could be identified by Western blot analysis with monoclonal antibodies against *M. arthritidis* and monoclonal antibodies against the large aggregating proteoglycans as proteins with molecular-weights between 22–65 kD. The cross-reactivity between *M. arthritidis* and rat chondrocytes appeared also on the cellular level. It was possible to establish $CD4^+$ T-cell lines responding to *M. arthritidis*, which could be stimu-

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lated by syngeneic chondrocytes in absence of *M. arthritidis* antigen. The reactivity of antibodies and T-cells with chondrocytes or proteoglycans, respectively, is discussed as an autoimmune phenomenon.

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K.14 Prolongation of allogeneic transplanted skin grafts by the anti-rat-TCR monoclonal antibody R73

H. U. Schorlemmer, E. J. Kanzy, R. Kurrle, and F. R. Seiler

The convincing success of a therapy protocol in kidney transplantation of the murine monoclonal antibody (mAb) directed to the human α/β-T-cell receptor (TCR) indicates that this mAb BMA 031 is not only immunosuppressive in acute rejection episodes but has also a long lasting immunoregulatory effect. BMA 031 is strictly human α/β-TCR specific and therefore all information about the mode of action derives from monitoring of patients or from *in vitro* experiments. The availability of a mAb directed to α/β-TCR of rats (generated by T. Hünig) allowed us to compare the *in vitro* and *in vivo* effects of this mAb with BMA 031. Here we evaluated the immunosuppressive efficacy of the anti-rat-TCR mAb-R73 in two experimental skin transplantation models: a) in the strong genetically defined system, leading to fast rejection (DA → LEW) and b) in delayed rejection (LEW → F344). Only two single intraveneous applications of mAb-R73 (0.5–5 mg/kg) caused a marked prolongation of graft survival time. In controls, graft rejection occurred about 10–12 days or 15–17 days after transplantation depending on the model used. When transplanted animals were treated with the anti-rat-TCR mAb-R73 more than doubling of the graft survival time was observed in both models. Prolongation of skin graft survival was also achieved when we used the $F(ab)_2$ fragment of the mAb-R73. When treatment with the mAb started in a very late stage of the experiment (during the rejection crisis) occasionally long-term graft survival was observed. With the anti-rat-TCR mAb-R73 we could show that in analogy to clinical short term application of BMA 031 the prophylactic application of MAb-R73 prolongs graft survival in a rat skin graft model.

Research Laboratories of Behringwerke AG, D-3550 Marburg/Lahn, FRG

K.15 Induction of tolerance by 15-deoxyspergualin in rat tail skin transplantation after combination therapy

H. U. Schorlemmer, R. Kurrle, and F. R. Seiler

The new immunosuppressant 15-deoxyspergualin (15-DOS) has shown efficiency in prolongation of graft survival time of different transplanted organs in several experimental allo- and xenotransplantation models. Also longstanding specific graft tolerance, which includes even skin grafts, has been reported. In general, however, induction of long-term graft survival

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in rats is a rare event for skin transplantation and has been observed only occasionally with 15-DOS monotherapy. The aim of this study was to elaborate whether induction of long-term graft survival or tolerance is possible even in skin transplantation and we tried to improve the therapeutic effectivity (treatment schemes) in acute rejection crisis by combination therapy experiments with cyclosporine A (CyA) and monoclonal antibody (anti-rat-T-cell receptor mAb-R73, generated by T. Hünig). For these experiments we used the two rat tail skin models with the MHC variant strain combination LEW → F344 or with DA → LEW rats. In these models, rejection of untreated controls occurs about day 15 or day 11, respectively, after engraftment. After a short-term application of 15-DOS or mAb-R73 or CyA, all drugs given prophylactically as monotherapy in an early stage after transplantation prolonged graft survival time in both models. However, when treatment started at the time of the expected rejection crisis only 15-DOS proved to be very effective. In combination therapy experiments with CyA or mAb-R73 the primary treatment with these drugs was discontinued to provoke rejection and then 15-DOS was given during the rejection period. Long-term graft survival for more than 100 days was observed. Subsequently in those long-term survivors second donor skin grafts were accepted as well without further drug treatment, whereas third party grafts were rejected. Therefore, 15-DOS not only represents a powerful rescue drug but also seems to develop specific tolerance after a short-term application during the rejection crisis.

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K.16 Therapy of human Multiple Sclerosis (MS): Disease modifying activity of 15-Deoxyspergualin on acute and chronic relapsing experimental Allergic Encephalomyelitis (EAE)

H. U. Schorlemmer and F. R. Seiler

EAE is an inflammatory and paralytic autoimmune disease of the central nervous system. Acute EAE generally is a monophasic disease that produces perivascular inflammation and sometimes small areas of demyelination. The chronic relapsing form of EAE in particular offers important similarities to the human disease MS. Due to its immunosuppressive mode of action, we examined the therapeutic effect of 15-deoxyspergualin (15-DOS) in the two models of acute and chronic relapsing EAE in Lewis rats. In the first model sensitization of adult rats (8–12 weeks of age) with guinea pig spinal cord in CFA results in an acute clinical episode of severe EAE and by day 16 the rats died. 15-DOS reduced the signs of the disease and prevented mortality. Even low amounts of the drug delayed and reduced the onset of clinical symptoms. The protection provided by 15-DOS was long-lasting and no subsequent relaps has been observed. Upon rechallenging, such convalescent rats were totally resistant to reinduction of the disease. In the second model of chronic relapsing EAE aged Lewis rats (6–8 months old) were immunized with myelin basic protein in CFA and additionally with *Bordetella pertussis*. Here all animals challenged developed a chronic relapsing EAE with up to three relapses. The second and third episodes were both milder and shorter in duration than the first. All animals treated with 15-DOS survived the first attack of the chronic disease which was also delayed in comparison to control. Complete recovery was evident one week after the first neurologic signs. None of the treated rats developed a relapse, a second or third episode was prevented, and all animals were able to sustain their remission indefinitely. These models demonstrate that they are well suited to the laboratory study of human MS, including its therapy in various stages of the disease.

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K.17 Platinum compounds induce pathological immune reactions in mice

H.-C. Schuppe[1], D. Haas-Raida[1], J. Kulig[1], U. Dickopp[1], E. Gleichmann[1], and P. Kind[1]

The potent sensitizing properties of certain platinum (Pt) compounds, such as hexachloroplatinate salts, are well known from Pt refineries. More than 50% of exposed workers develop symptoms of type I hypersensitivity, including bronchial asthma. Although unconvincing attempts to demonstrate Pt-specific IgE have been made, the precise mechanism of sensitization is still unclear. Similar to other heavy metal salts not only «allergic» but also «autoimmune» reactions have to be considered. In this study, for the first time immunopathological reactions to Pt compounds were shown in mice. C57BL/6 and B10.S mice received s.c. injections, with 0.4, 2, 10 µg Na_2PtCl_6 or NaCl three times weekly throughout the experiment. Sera were analysed for the induction of antinuclear autoantibodies (ANA) by indirect immunofluorescence. Within 14 weeks, 3/8 C57BL/6 mice treated with 0.4 or 2 µg developed ANA. Positive sera revealed a homogeneous staining pattern in dilutions between 1:10 and 1:320. All B10.S mice remained ANA-negative. A 2- to 5-fold increase of serum IgE was inducible in C57BL/6 mice within 10–20 weeks when Na_2PtCl_6-treatment (0.4 µg) was combined with monthly i.p. injections of $Al(OH)_3$. The single substances showed no adjuvant activity for IgE production. The local sensitizing potential of hexachloroplatinate salts was determined using the popliteal lymph node (PLN) assay. In various mouse strains except athymic nude mice Na_2PtCl_6 or $(NH_4)_2PtCl_6$ induced a dose-dependent increase in PLN weight (up to 4.5-fold) and cell number (up to 10-fold) when injected into one hindfoot pad. Maximal results were shown on day 6 after 40 µg $(NH_4)_2PtCl_6$. A specific secondary response could be elicited with subimmunogenic doses after 5–7 weeks. In conclusion, both local and systemic immunopathological reactions to Pt salts are inducible in mice thus providing a useful animal model. It is suggested that Pt-specific T cell sensitization plays a central role for these graft-versus-host like reactions.

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K.18 Contact sensitivity to $HgCl_2$ in mice

C. P. Stringer[1], P. Kind[2], and E. Gleichmann[1]

Systemic treatment with sub-toxic doses of $HgCl_2$ induces high serum levels of IgE and IgG_1 in certain mouse strains (e.g., ASW, but not in DBA/2). We have previously shown that this induction requires IL-4, which could be released from Hg-specific T_H2 cells. We investigated contact sensitivity (CS) to $HgCl_2$ in DBA/2 mice in order to establish whether Hg could stimulate a T_H1 cell-mediated response. Mice were sensitized on the flank and CS was assessed by measuring ear swelling with an engineer's micrometer after ear-challenge. Significant ear swelling was detected in $HgCl_2$-sensitized animals only and was Hg-specific. The peak ear swelling reaction occurred 4 days after challenge. We are currently investigating the nature of

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this late CS reaction, which from preliminary results appears to be under MHC control. Furthermore, as delayed-type hypersensitivity is T_H1 cell-mediated, by studying the pattern of responsiveness to Hg-induced IgE/IgG_1 or CS in several mouse strains, we are also determining whether Hg preferentially activates T_H1 or T_H2 cells depending on the MHC haplotype.

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K.19 The hepatic asialoglycoprotein receptor as target for autoreactive immunity

U. Treichel, H. Löhr, T. Poralla, M. Manns, G. Hess, B. Fleischer, and K.-H. Meyer zum Büschenfeld

A number of autoimmune diseases are characterized by autoimmune reactions directed against enzymes or receptors of the target cells. In autoimmune chronic hepatitis (ai-CAH) we could demonstrate that the affinity-purified human asialoglycoprotein receptor (h-ASGPR) is recognized by both humoral and cellular immune responses. In man, autoantibodies directed against the h-ASGPR (anti h-ASGPR) were detected in 88% of patients with inflammatory active ai-CAH in contrast to only 5% of patients with viral hepatitis. During inflammation anti h-ASGPR were of both IgG- and IgM class, in treatment induced remission exclusively of IgG class, in rare cases of anti h-ASGPR positive viral hepatitis always of both IgG-/IgM class. Immunomodulation of chronic active hepatitis B by alpha-INF induced anti h-ASGPR only transiently and in low titer. Immunosuppressive therapy of ai-CAH markedly reduced anti h-ASGPR titer in all patients. 34% of peripheral blood lymphocytes (PBL) of patients with ai-CAH specifically proliferated in the presence of h-ASGPR in contrast to 0% PBL of patients suffering from viral hepatitis. Additionally, only the PBL of patients with ai-CAH were capable to secret anti h-ASGPR *in vitro*. Moreover, we have successfully established liver-derived T-cell clones (mainly of CD4 phenotype) from patients with ai-CAH which proliferated specifically and HLA-restricted in the presence of h-ASGPR. In summary, the h-ASGPR seems to represent an important target for humoral and cellular autoimmune mechanisms in chronic liver diseases.

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Workshop L
Clinical Immunology

Forschungsinstitut Borstel, Parkallee 22, D-2061 Borstel, FRG

L.1 IgG subclasses in asthma bronchiale and chronic obstructive bronchitis

S. Anders, K. Löck, H. Haas, and M. Schlaak

In the pathogenesis of obstructive airway diseases the role of bacterial and viral infections as an initiating and maintaining factor is discussed. The exact pathway of airway inflammation and the involvement of humoral immunomechanisms are unknown. Since deficiencies of certain IgG subclasses are known to be associated with a higher frequency of airway infections, we investigated the sera of 99 patients with asthma (52) and chronic obstructive bronchitis (47) for IgG subclass deficiencies. IgG subclass levels were measured by means of a dot-immunobinding assay (DIBA).

Results: Significantly lower levels of IgG1, 2 and 3 were found in patients with asthma bronchiale or chronic obstructive bronchitis as compared to healthy blood donors. In 26 of 99 patients (26,3 %) one or two subclasses were below the normal range, while no patient showed a complete absence of any subclass. Patients with decreased IgG subclass levels also had lower levels of IgA and IgE, and were more often treated with corticosteroids than the other patients. In this context, it remains to be settled if corticosteroid therapy itself has a reducing effect on IgG subclass levels.

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L.2 CD4-negative dendritic B-cell lines are susceptible to HIV-1 infection

Rudolf Berger, Reinhard Gillitzer, Klaus Wolff, and Georg Stingl

We have recently generated two dendritic B-cell (DBC) lines by Epstein-Barr virus (EBV) transformation. These B cells resemble dendritic cells with regard to their phenotype and their extremely high stimulatory capacity in the allogeneic mixed leukocyte reaction.

Since follicular dendritic cells are targets for HIV-1 *in vivo*, we were interested to know, whether DBC can be infected by HIV-1. Despite the fact that both cell lines do not express

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CD4 at the RNA and protein level, DBC's could be readily infected by HIV-1. Virus replication in DBC (as judged by p24 antigen ELISA) was comparable to p24 levels in HIV-1-susceptible T-cell lines. Preincubation of target cells or HIV-1 with monoclonal anti-CD4 antibodies or soluble CD4 at concentrations that abolished infection of T-cell lines with HIV-1 had no effect on the infection of DBC.

These results suggest that cell surface molecules different from CD4 are relevant for virus entry into this particular cell type.

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L.3 Characterization of the $CD3^+$ $CD8^+$ $CD57^+$ T-cell-subset expanded in patients with longtime immunosuppression and virus infection

W. D. Docke[1], B. Reipert[5], A. Lukowsky[1], P. Reinke[2], H. Schüler[4], H. Wolf[3], H. Warnke[3], K. Neuhaus[1], and H. D. Volk[1]

The lymphocytes of renal, liver and heart long-term (>6 months) allograft recipients (transplanted in 3 different centers) were cytofluorometrically analysed in follow-up studies. About 20% of renal graft recipients and 65% of heart graft recipients showed an increased proportion and number of $CD8^+$ T cells with the phenotype $CD3^+$ $CD8^+$ $CD57^+$. A high proportion of these cells is activated and expresses the HLA-DR antigen. This phenomenon is stable over months and follow-up studies (2–3 years post Tx) have shown that in most cases the expansion of this $CD8^+$ subset has been observed between the 6th and 12th month post Tx. A striking, long-lasting expansion of this $CD8^+$ $CD57^+$ subset occurred in 2 liver allograft recipients suffering from acute systemic CMV infection. An association between chronic virus infection (esp. CMV) and rejection processes was presumed by several authors. For further characteration of this $CD3^+$ $CD8^+$ $CD57^+$ T-cell subset multiparameter flow cytometric studies were performed, T-cell receptor repertoire was investigated by using V_H-TCR family restricted mAb and Southern-blot technique, immunoregulatory (help/suppression of immunoglobulin synthesis) and cytotoxic function assays were carried out. Experiments are in progress to study the lymphokine pattern (mRNA) of this $CD8^+$ T-cell subset.

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L.4 Reduced intracellular glutathione levels in HIV-1 seropositive persons

Hans-Peter Eck and Wulf Dröge

We have previously reported that all groups of HIV-1 infected persons including persons without overt symptoms and persons with lymphadenopathy syndrome express a metabolic

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disorder that is characterized by elevated plasma glutamate and strongly reduced plasma cysteine levels. Both effects are expected to impair the supply of cysteine to immunologically relevant cells, since glutamate is known to compete with cystine for the same membrane transport system. It is also known that intracellular glutathione levels are important for lymphocyte functions. We, therefore, investigated the intracellular glutathione levels in peripheral blood mononuclear cells and monocytes of HIV-1 infected patients. Our studies revealed that the intracellular glutathione concentration is significantly decreased in the peripheral blood mononuclear cells and monocytes of HIV-1 seropositive persons with lymphadenopathy syndrome and in patients with AIDS. Complementary laboratory studies with buthionine sulfoximine, a specific inhibitor of glutathione biosynthesis, showed that even a moderate reduction of the intracellular glutathione level markedly affects the proliferative activity of mitogenically stimulated lymphocytes and cloned T cells. A metabolic defect in HIV-1 infected patients may contribute to the pathogenesis of the disease by reducing the intracellular glutathione levels. This effect appears to have consequences for lymphocyte functions. Since independent studies by Mihm and Dröge have shown that even a moderate reduction of the intracellular glutathione level significantly alters the DNA-binding activity of NFκB-like nuclear proteins in various human and murine cell lines, it appears that viral activity is also influenced by reduced glutathione levels.

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L.5 Flow cytometric and immunohistological investigations of lymphocyte populations in long term renal allograft recipients. Detection of CMV-coded antigens and CMV-DNA by immunohistology and *in situ* hybridization

Ellen Fietze[1], D. Volk[1], Petra Reinke[2], G. Niedobitek[4], Teresa Finn[4], G. Ditscherlein[2], Kristin Neuhaus[1], and H. Stein[4]

Tissues and blood samples from 80 patients, recipients of cadaveric renal allografts at least 6 months previously and hospitalized for various complications were investigated. Mononuclear cells of the peripheral blood were studied for activation and differentiation markers by flow cytometric analyses. Graft biopsy infiltrates were characterized morphologically and immunohistologically using the APAAP-method and correlated to FACS-analysis results in peripheral blood. An association between chronic virus infection and the rejection process was suggested by several authors. Therefore we investigated the graft biopsies of 10 patients for the expression of CMV-encoded antigens and CMV- and EBV-DNA by APAAP-immunohistology and *in situ* hybridization using radiolabelled probes. No evidence of CMV infection was found in any of the biopsy specimen, while positive control sections displayed a strong labelling with both techniques.

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L.6 High *in situ* expression of IL-6 and IL-8 mRNA in Kaposi's sarcoma (KS): a possible role in KS tumorgenesis

Reinhard Gillitzer, Georg Stingl, Klaus Wolff, and Rudolf Berger

Immunohistological and ultrastructural studies have shown a multitude of different cell types (endothelial cells, fibroblasts, dermal dendrocytes (DD), lymphocytes, and so called KS cells), comprising KS lesions. Particularly, KS cells and DD are the dominating cells in KS. Using immunophenotyping, we identified the following antigenic pattern: KS cells (EN-4^{++}, PAL-E^{+}, vWF$^{+/-}$, 44G4^{+}, UEA^{++}, BMA120^{++}, laminin^{++}, collagen IV^{++}, HLA-DR^{++}, ICAM-1/2^{-}, ELAM-1^{-}, ENA-1^{-}), DD (XIIIa^{++}, KiM7^{++}, KiM8^{++}, HLA-DR^{++}, CD14^{+}, CD1^{-}, CD3^{-}). Since growth factor secretion may play a major role in the initiation of KS and/or subsequent tumor cell growth, we performed RNA *in situ* hybridization to address this issue. Strong IL-6 mRNA expression was predominantly localized in cells situated around and partly within KS lesions. Furthermore, abundant IL-8 mRNA expression was found in the regions adjacent to KS lesions, thus partly overlapping the area of IL-6 mRNA expression. The region of high IL-6/IL-8mRNA expression corresponds to the area of tumor expansion. Therefore, it is tempting to speculate that IL-8 secretion caused by KS cells stimulates DD to secrete IL-6 as well as other angiogenic factors, essential for KS growth. The role of other angiogenic factors in tumor growth *in situ* is currently being investigated.

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L.7 *Post mortem* MHC class I and II typing on retinal epithelial cells

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The aim of this study was to establish a quick and reliable technique by which cadaver can be HLA typed. Retina epithelial cells were obtained from cadaver bulbi 8–36 h *post mortem*, n = 19. In 7 cases, cells were obtained > 22 h *post mortem*. The rate of cell growth was significantly dependent on the time lapse between donor's death and bulbi explantation with subsequent cell cultivation. Cells were stimulated by different gamma-IFN concentrations and examined by FACS analysis for the expression of MHC class I and class II molecules. 100 U/ml gamma-IFN were sufficient to induce maximal class I expression by > 85–90 % cells after 3 days. At 250 U/ml IFN, only 30 % of the cells showed maximal class II expression. Maximal expression of class II molecules was not achieved 7 days post stimulation with 750 U/ml IFN. Serological HLA typing of both class I and II specificities was possible using the standard lymphocytotoxicity assay 3 days post gamma-IFN (250 U/ml) stimulation. Unclear serological results on class I specificities were clarified by ID-IEF, enabling full HLA typing on all donor bulbi examined. Results were equally good when 500 U/ml gamma-IFN were used for cell stimulation. In contrast to claims by others, only 2–3 class I specificities per donor cells were clearly identifiable on serological typing 5–7 days post stimulation with 750 U/ml. Class II specificities were not difficult to identify at all three concentrations. Our results indicate that successful HLA typing of RPE is dependent on both gamma-IFN concentrations used and the number of days cells are stimulated prior to typing. Furthermore, we show for the first time successful serological typing of class II specificities on RPE, thus providing a strongly improved basis for cadaver cornea grafting.

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L.8 Elevated serum levels of soluble CD14 in patients with insulin dependent diabetes mellitus (IDDM)

C. Krüger, Elke Gens, D. Michaelis, and Christine Schütt

The soluble form of the monocyte surface glycoprotein CD14 (sCD14) is present in normal human serum (6.7 ± 0.8 µg/ml). In 43 young diabetics suffering from type I diabetes (IDDM) we studied the sCD14 content in serum. During hospitalizations for dietary and therapeutic optimization of metabolic conditions we estimated sCD14 levels using a capture ELISA (sCD14-ELISA, IBL, P.O. Box 2808, 2000 Hamburg, FRG) in comparison to clinical parameters (hyperglycemia, HbA_1-content, C-peptide etc.).

In patients with poor metabolic situation at admission to hospital we found the highest sCD14 levels (16 µg/ml). During successful dietary and therapeutic intervention the sCD14 content decreased drastically within 1–3 weeks. In cases of unsuccessful therapeutic management with remaining metabolic dysfunctions the elevated sCD14 levels did not decrease. The results demonstrate the involvement of monocytes in the pathogenesis of the autoimmune disease, which seems to be important especially for the development of late complications.

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L.9 Elevated levels of soluble CD14 molecules in serum of HIV-1 infected patients

C. Krüger, Christine Schütt, H. Dietz, Jane Lüdtke, B. Ruf, Angelika Langford, H. D. Pohle, and R. O. F. Kunze

CD14 is a well known monocyte surface marker which exists in a membrane bound and a soluble form (sCD14). In normal human sera sCD14 ranges from 6.0–7.5 µg/ml. We investigated sera from HIV-1 infected patients in/during different clinical stages and antiviral therapies. sCD14 were determined by a capture ELISA (sCD14-ELISA, IBL, Hamburg, FRG). In addition to this new parameter HIV-relevant immunological standard parameters β_2-microglobulin, neopterin and $CD4^+$ and $CD8^+$ peripheral blood lymphocytes were assayed. In the clinical stages LAS and ARC elevated levels of sCD14 up to 12 µg/ml were observed. The highest values for sCD14 we found in sera of patients with full blown AIDS (18 µg/ml). sCD14 varied independently from the immunological standard parameters. Although nothing is known about the function of CD14 on monocyte surface and sCD14 the results demonstrate the involvement of the CD14 glycoprotein in the immunopathogenesis of HIV-infection. The usefulness of this test to indicate different types of opportunistic infection (viral/bacterial) is suggested and under investigation.

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L.10 Phenotypical and functional assessment of T cells from patients with chronic T cell leukemia

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T cells of four patients with chronic lymphocytic leukemia of the T-cell type (T-CLL) were investigated. Three of the leukemic clones expressed αβ T-cell receptors (TCR), whereas one was γδTCR-positive. αβTCR$^+$ clones did not express variable regions that could be detected by available monoclonal antibodies (mAb) against Vα2, Vβ5, Vβ6, Vβ8, or Vβ12 determinants. Two of the αβ$^+$ clones were CD4/CD8 doublepositive, one was CD4+CD8$^-$. IL-2-receptor expression was detectable on all αβ$^+$ clones after stimulation with mitogens such as phytohaemagglutinin (PHA). The γδTCR$^+$ clone probably expressed $V_δ1$ as variable region. The TCR of this clone could be stained with mAb δTCS1 and TCRδ1, but not with mAb TiγA and 7A5. Functional analyses of the proliferative and helper (i.e. IL-2 producing) capacity of leukemic T cells revealed that two of the αβ$^+$ leukemic clones strongly responded to TPA, PHA, and ConA. In contrast, the third αβ$^+$ (CD4$^+$/CD8$^+$) clone showed a moderate spontaneous proliferation which was suppressed by addition of phorbolester and/or Ca^{++} ionophore. The γδ$^+$ clone did not show any significant proliferative response to various stimuli tested so far. T cells of all patients were not inducible to kill P815 target cells in the presence of anti-TCR/CD3 mAb.

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L.11 Intracellular glutathione level controls DNA-binding activity of NFκB-like protein(s)

Sabine Mihm and Wulf Dröge

The LTR region of the HIV-1 genome contains enhancer sequences including the κB-sequence that is known to regulate light chain expression in B-cells. Since we have previously found that HIV-1 infected persons have generally reduced plasma cysteine concentrations and decreased intracellular glutathione levels, we have now investigated the influence of the intracellular glutathione concentration on the regulation of HIV-1 gene expression. Here we report the effect of intracellular glutathione levels on DNA-binding proteins that bind to one of the enhancer sequences of the HIV-1 genome. The intracellular glutathione level was experimentally elevated by administration of glutathione to the medium and decreased by administration of buthionine sulfoximine, a specific inhibitor of the glutathione biosynthesis. Band shift assays were used to determine the DNA-binding activity of nuclear protein extracts to radioactively labeled κB-sequences. In several different human and murine cell lines we observed that even a moderate decrease of the intracellular glutathione level increases the DNA-binding activity of NFκB-like proteins. Our studies strongly suggest that the reduction of intracellular glutathione levels in HIV-1 infected persons contributes to the regulation of viral activity in these patients.

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L.12 *In vitro* studies on cellular immunity in adult patients with chronic bronchitis

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The immunological status of 30 patients with chronic bronchitis (WHO-criteria), 17 receiving systemic corticosteroid (>5 mg/day prednisolon) treatment, was evaluated and compared with 12 age matched controls. In addition to serum levels of IgA, IgM, IgG as well as IgG subclasses we determined lymphocyte subsets CD2, CD3, CD4, CD5, CD8, CD16, CD19, CD20, leu7 by flow cytometry as well as lymphocyte response (^{3}H-thymidin incorporation) to mitogen (PHA, ConA, PWM), tetanus-antigen and the monoclonal antibody anti-CD3.

Compared to the controls lymphocyte transformation induced by PHA ($p < 0.001$), ConA ($p < 0.001$), anti-CD 3 ($p < 0.001$) and tetanus-antigen ($p < 0.05$) was significantly lower in both patient groups whereas between both groups there were no significant differences. Experiments with PWM stimulation did not show significant differences. The same was true for the distribution of all lymphocyte subsets between the three groups. As expected, the steroid treated patient group had lower serum IgG levels ($p < 0,03$) than the group without steroid medication and the controls. No patient was found to be IgG subclass deficient.

Our data indicate, that in chronic bronchitis patients cellular immune responses to PHA, ConA, antigen and monoclonal antibodies are reduced which is not related to corticosteroid medication.

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L.13 Regeneration of autologous transplanted lymph node fragments

H. J. Rothkötter and R. Pabst

Autologous avascular lymph node fragments have the capacity to regenerate, possibly a future technique for clinical treatment of lymphoedema [1]. It was examined whether the source of the transplanted fragments or the site of implantation influences the regeneration. In two month old minipigs the superficial inguinal (iLN) and a part of the mesenteric lymph node (mLN) were excised. In 27 pigs thin slices of these nodes were avascularly implanted as autologous grafts in two ways: subcutaneously mLN-fragments in the left or iLN-fragments in the right groin region or iLN-fragments either subcutaneously in the groin region or subfascially in the thigh. Grafts, excised 2 and 3 weeks after implantation showed fatty degeneration. From 3 weeks after transplantation onwards groups of lymphocytes were observed in the degenerated tissue, partially showing follicle-like structures. In the follicles mitoses were observed. Six months later regenerated tissue was found in 32 of the 76 subcutaneous iLN-implants, and in 6 of the 60 mLN-implants. However, only 2 of the 14 subfascial implants regenerated. Immediately before death subcutaneously injected Berlin blue was detected in the sinus of the small nodes indicating restored lymphatics. After injection of inactivated microbial antigen in the draining area of the graft, germinal centre formation was observed in the regenerated tissue. In the paracortex, high endothelial venules indicated the

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normal microenvironment for lymphocyte migration. After fatty degeneration, the implants showed lymph node structure with all compartments, the amount of regeneration depending on the source of the grafts and the site of implantation. Perhaps this method can initiate formation of new lymphatics in patients with lymphoedema.

1. PABST, R., and ROTHKÖTTER, H. J. 1988. Cell Tissue Res 251: 597.

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L.14 Cytokines in the diagnosis of renal allograft rejection

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Rejection episodes and viral infections are major complications of organ transplantation. To examine whether selected cytokines could represent ueful diagnostic tools to differentiate deteriorating graft functions, we determined interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α) and soluble interleukin-2 receptor (IL-2R) by highly specific immunoassays. In parallel, neopterin and serum amyloid A (SAA) were determined. 14 patients were included, 10 acute rejection periods occurred, 3 patients showed a herpes virus infection, 4 patients received an ATG and 6 on OKT3 mAb therapy. In all patients, steroids and cyclosporine A were used routinely. While increased levels of SAA and neopterin in plasma correlated well with acute rejection periods, it was also found that high neopterin concentrations in urine were indicative of a viral infection. Compared to these established parameters, the determination of cytokines did not show a consistent pattern. In most cases, plasma levels of TNF-α and IL-2R were moderately elevated during rejection episodes. During viral infections, however, the concentrations of IL-2R were much higher, particularly in urine. Interestingly, IL-1β was only found in patients with ATG therapy. In conclusion, TNF-α and IL-2R in combination with SAA and neopterin may represent helpful markers in establishing a differential diagnosis of acute allograft rejection and viral infections. In contrast to published reports, reproducible and clear pattern of cytokine release was not observed which may be partially due to immunosuppressive therapy.

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L.15 T4a-specific antibodies imitating the gp120 binding site used for vaccination against HIV

C. SUTOR, U. DREIKHAUSEN, and I. SCHEDEL

Monoclonal antibodies generated against the T4a epitope were shown to block gp120-CD4 interaction and inhibit syncytia formation in HIV-infected lymphocyte cultures. These T4a-specific antibodies may serve as a vaccine to raise – via an anti-idiotypic response – antibodies cross-reacting with the gp120 binding site and possibly inhibiting HIV-infection. Rabbits have been vaccinated with different T4a-specific mouse-monoclonal antibodies. The IgG-fractions were isolated from the immune sera and absorbed with whole mouse IgG to remove all

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reactivity against mouse IgG isotypes. Serum titres and anti-idiotypic activity were measured in an ELISA. The different purified anti-idiotypes reacted – even at high dilutions – with the idiotypic region of the corresponding monoclonal antibody used for vaccination. Strong crossreactions were seen with anti-CD4 antibodies recognizing overlapping epitopes but not with irrelevant monoclonal antibodies as determined by FACS analysis. An ELISA designed to measure anti-gp120 antibodies revealed specific reactivity of some anti-idiotypes to the HIV-1 surface glycoprotein. Cell tests for detecting *in vitro* neutralization of HIV are currently being done.

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L.16 High dose intralesional recombinant interferon-alpha-IIb-treatment in HIV-1-infected patients with Kaposi's sarcoma

B. Tschechne, P. von Wussow, I. Schedel, and H. Deicher

Seven patients with histologically proven KS and HIV-1 infection have been treated by intralesional injections of IFN-α-IIb, 3×10^7 IU per dose daily for a period of 4 weeks, followed by a reduced dosing of 3×10^7 IU per dose 3 times weekly. The correlation of clinical course and different biological parameters, HIV-antigen (Ag) level, IFN-antibodies, Mx-protein as well as clinical response were assessed. The treated tumor lesions were clinically compared (size, colour, shape) with non-treated nodes. All treated KS-nodes were noted to be diminished in cutaneous thickness, tumor size and intensity of colour. Patients classified as KS without opportunistic infections (O.I.) showed a complete remission in 2/5 cases, whereas 2 patients with KS and O.I. did not show any clinical response. Patients with isolated cutaneous KS had a higher frequency of a clinical response than those who had visceral lesions (2/4). Patients who had a pretreatment value of total lymphocyte counts (> 3000/μl), CD4 counts (> 400/μl) and CD4/CD8-ratio (> 0.4) reached a higher chance of clinical response. An effect was observed in 1/3 of HIV-AG negative group, in 1/4 of the HIV-AG positive group. In the HIV-AG positive group responding to IFN-α-IIb-treatment 2/4 became AG negative. AIDS-related KS responded in 57% to intralesional application of IFN-α-IIb. In addition, patients with KS without O.I. as well as CD4 lymphocyte counts > 400/μl and without visceral KS lesions may have a superior effect.

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L.17 Bone marrow cell number plays, but GvHR-induced immunosuppression does not play a role in preventing graft rejection after allogeneic bone marrow transplantation (BMT)

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We have investigated the impact of pretransplant immunosuppression, BM cell dose and GvHR on the engraftment of MHC-allogeneic marrow grafts. LEW rats received a lethal dose

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of busulfan prior to BMT. Since busulfan is not sufficiently immunosuppressive, allogeneic marrow grafts are rejected unless further immunosuppression is administered. Hematocrit and granulocyte counts were monitored for each animal and rejection was defined as death with granulocytes $< 500/\mu l$. Surviving animals received a donor type skin graft to confirm persistence of allogeneic hematopoiesis. In strictly parallel experiments we varied (1) the number of bone BM cells transfered (1, 5, 10, 20, and 40×10^7 i.v.), (2) the degree of immunosuppression (0, 30, 60, 90, 120, and 180 mg/kg cyclophosphamide (CY) i.p. day -2/-3), and (3) the ability of the marrow graft to induce a GvHR [(CAPxLEW)F1 or CAP rats as BM donors]. Reducing either the number of BM cells or the dose of CY resulted in a stepwise increase of rejection rates. However, each reduction of the BM cell number could be compensated by higher doses of CY and vice versa. No rejections of (CAPxLEW)F1 grafts were observed after at least 60 mg/kg CY and 40×10^7 BM cells, 90 mg/kg CY and 20×10^7 BM cells, 120 mg/kg CY and 5×10^7 BM cells, 180 mg/kg CY and 1×10^7 BM cells. Engraftment of syngeneic marrow was seen following cell numbers of as low as 5×10^6. CAP marrow, in contrast to (CAPxLEW)F1 marrow, can lead to GvHR-mediated immunosuppression in LEW recipients. Therefore, we had supposed that lower cell numbers or CY doses are sufficient to achieve engraftment of CAP grafts. However, although severe GvHR was present in all of the animals receiving escalating doses of CAP cells, the rejection rates were the same as for (CAPxLEW)F1 marrow. In summary, we have demonstrated a sensitive balance between immunosuppression and BM cell number. Our data do not support the hypothesis that the loss of GvHR-mediated immunosuppression is responsible for higher rejection rates following T-cell depleted BMT.

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L.18 Effective natural interferon-alpha therapy in recombinant interferon-alpha antibody positive hairy cell leukemia patients

P. von Wussow, D. Jakschies, H. Pralle, R. Sonnen, and H. Deicher

83 non-splenectomized hairy cell leukemia patients were treated in a prospective randomized trial with 1.2 Mill. IU IFN-α2a/m^2 either daily or cyclically from day 1 to day 7 and screened for emergence of interferon (IFN) antibodies in their serum. 15 patients developed IFN-binding and 9 IFN-neutralizing antibodies, which recognized equally well recombinant IFN-α2a, b, c, but only marginally natural IFN-α. 5 of 15 IFN antibody positive patients experienced deteriorating peripheral blood cell counts despite continuous rIFN-therapy, while not a single relapse of the disease was observed in IFN antibody negative patients. 7 of the 9 recombinant IFN non-responsive and rIFN antibody positive patients were switched to natural IFN-α at a dose of 3×3 Mill. IU per week. All 7 patients responsed to nIFN-α achieving either partial or minor hematological remissions. While after rIFN-α2 injections significantly altered IFN kinetics and only minimal Mx-homologue, an intracellular IFN-induced protein, concentrations in PBL were measured in rIFN-α positive patients, natural IFN-α injections led to normal IFN serum levels and dose-equivalent Mx-homologue amounts. These data prove that high titered recombinant IFN-α neutralizing antibodies can abrogate the biological action of recombinant IFN-α but not of natural IFN-α and that natural IFN-α can effectively overcome the acquired IFN resistance due to rIFN-α antibodies in hairy cell leukemia.

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L.19 Increased C3a-desArg concentration as indicator for neonatal infections

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In order to establish informative diagnostic parameters, complement activation products were determined in EDTA-plasma of normal neonates and in neonates with various diseases in a prospective study. Cord blood and blood obtained by venipuncture were compared. Gestational age of term and preterm neonates ranged from 31–42 weeks. The anaphylatoxin C3a-desArg was determined with a novel ELISA system using a monoclonal antibody reacting with a neoepitope of C3a-desArg. The C1rsC1Inactivator complex generated upon activation of the classical pathway and the C3b(Bb)P complex generated upon activation of the alternative pathway were measured by double sandwich ELISA systems using commercially available antibodies. C3a-desArg levels in cord blood of normal neonates were markedly diminished (85 ± 64 ng/ml) compared to blood obtained by venipuncture, whereas there were no differences in C3, C1rsC1Inactivator and C3b(Bb)P levels. There were no differences between plasma concentrations of C3a-desArg in neonates (180 ± 91 ng/ml) and a control group of normal adults (171 ± 76 ng/ml) whereas diminished C3 levels (0.65 ± 0.14 mg/ml) were found in neonates as reported in the literature. Neonates with slight perinatal asphyxia had C3a-desArg levels within the normal range, but neonates with severe perinatal asphyxia showed slightly elevated C3a-desArg levels. In contrast, in neonates with amniotic infection, with pneumonia or sepsis a significant initial increase of C3a-desArg was observed. In parallel an increase of the activation product C3b(Bb)P was found due to alternative pathway activation, whereas C1rsC1Inactivator complex remained within the normal range. These data indicate that marked increase of C3a-desArg concentration is a specific indicator for severe neonatal infection.

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